Characterizing the Mechanical Parameters of the Fission Yeast Cell Wall Using Atomic Force Microscopy

by

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Approved by
Supervising Committee:

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For

My Family (Mom, Dad, Achsah)

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Abstract

While the biological and chemical aspects of the yeast cell have been studied in great depth, much less is known about their physical parameters. The fission yeast cell wall is a dynamic structure that is subject to variable internal forces during the cell division cycle. Since fission yeast (*Schizosaccharomyces pombe)* is used frequently in biology as a model organism to study higher order eukaryotes, we decided to examine the physical properties of their cell walls. Using an Asylum MFP3D Atomic Force Microscope, we collected force vs. indentation graphs and fit them to exponential and Hertz models using Igor Pro software. We took multiple curves at a single point on the cell wall to check for the precision of our measurements, and also examined different cells at different points along the cell wall. We found a characteristic length τ for each curve using our exponential model, and an approximate value for the Young's modulus of the cell using a Hertz model. We found an average value of 41.1 ± 7.9nm for τ after examining a total of 51 curves. For the elastic moduli, we found a striking difference between those of cells grown to a normal optical density (OD) and those grown to a low OD. The average elastic modulus of cells grown to normal concentrations was 7.9 ± 4 MPa, while that of the undergrown cells was only 40.1 ± 10 kPa. We concluded that the cell wall displays a nonlinear response to stress, likely because it is composed of multiple polymer layers.

Introduction

Motivation

While, the qualitative features of biological systems have been studied in depth, the mechanical properties of these systems remain poorly understood. Describing a biological process, such as the cell cycle or cell-receptor interactions, is much less involved than quantifying the forces and physical parameters involved. However, a purely qualitative analysis does not allow for a complete understanding of the systems being observed. In the words of nineteenth-century physicist Lord Kelvin,

> "In physical science, the first essential step in the direction of learning any subject is to find principles of numerical reckoning and practicable methods for measuring some quality connected with it. I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind." [9]

The underlying goal of biophysics is to quantitatively answer questions about living systems on all levels of biological organization. This interdisciplinary science integrates multiple fields including nanotechnology, bioengineering, and biochemistry to numerically describe the features of a biological system.

In our experiments, we chose to investigate the fungus *Schizosaccharomyces pombe (S. pombe)*, also known as fission yeast. These cells excellently model higher order eukaryotic cells, but are easier to culture and manipulate than their mammalian counterparts. The two main goals of this project are to quantify the strength of the yeast cell wall by finding the force constants and elastic moduli at different points along the cell, and furthermore to characterize the forces exerted on the cell wall during mitosis. My research deals with the first of these goals, and further experimentation is needed to

quantify the latter. I will detail the methods we used to characterize the physical parameters of the cell wall, and briefly mention potential future work on this project in the concluding section.

Background

Regardless of its outward appearance, a eukaryotic cell is a highly complex space with many specialized components. In a miniscule 5-10 μm length, the cell contains the parts required to sustain its existence, and the machinery needed to replicate and pass genetic information on to future generations. Fungi are a special class of eukaryotes that shares features with both plant and animal cells. Fungal cells have a rigid cell wall composed of chitin and polysaccharides, along with a nucleus surrounded by a nuclear membrane. I will describe in detail two structures, the fungal cell wall and the cytoskeleton, which are particularly relevant to my research.

The Cell Wall

The cell wall provides a cell with structural support and protection, and prevents over-expansion of the cell when water enters. The integrity of this structure is particularly important to yeast because fungal cells maintain a high turgor pressure during their existence. Detailed chemical analysis of the yeast cell wall has shown that it is composed of four primary structural components: α- glucan, β-glucan, chitin and manoprotein[8]. Unlike those of insects and plants, the fungal cell wall is a dynamic system that is constantly remolded to meet the cell's needs. Consequently, many cell wall-associated proteins are enzymes which hydrolyze chitin and polysaccharides. Because of this constant activity, the biosynthesis of cell wall components involves numerous regulatory pathways that are difficult to understand[8]. For our purposes however, we are not

concerned with the biochemical changes affecting the cell wall but with its mechanical parameters. Of particular interest are the forces acting on the cell wall during mitosis. During mitosis and cytokenisis, a cleavage furrow forms between two yeast daughters cells and new cell wall materials are deposited at this site. This results in septation, the formation of a new cell wall between the daughter cells. During mitosis, the cell wall is involved in a complex interplay with members of the cytoskeleton, in particular microtubules, which provide the tracks along which membrane vesicles are transported to the site of cleavage[3]. We highlight the role of these structures in the following sections.

The Cytoskeleton

The ability of a cell to interact with its microenvironment is governed by the cytoskeleton, a filamentous network located inside a cell's cytoplasm. This network is responsible for helping the cell maintain physical robustness, proper shape, and internal structure. The cytoskeleton is composed of three primary filaments: actin, intermediate, and microtubule filaments. Actin filaments are composed of actin monomer subunits and play major roles in cell locomotion and cell surface determination. Intermediate filaments provide mechanical strength and resistance to shear stress, and microtubules determine the location of internal organelles and direct intracellular transport. The importance of microtubules in the cell division cycle makes them a chief topic for investigation.

Microtubules

Microtubules are rigid hollow rods approximately 25 nm in diameter that undergo continual assembly and disassembly in the cell[2]. They are composed of the globular protein tubulin, which is a dimer consisting of two closely related 55-kd polypeptides, α-tubulin and β-tubulin. As shown in Figure 1, tubulin dimers polymerize to form several

protofilament arranged around a hollow core. Like actin filaments, microtubules are polar structures with a fast-growing end and a slow-growing end. This property plays an important role in determining the direction of movement along a microtubule.

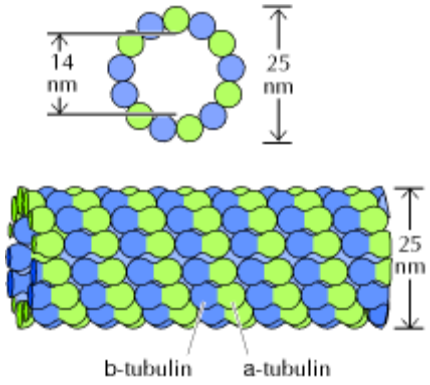


Figure 1 Structure of Microtubules[2]

An important feature of microtubules is dynamic instability, the altering of individual microtubules between cycles of growth and shrinkage. Microtubules have both α and β tubulin ends which can bind to guanosine triposphate (GTP), a high energy molecule used in numerous biochemical reactions. In the cell, GTP bound to the β-tubulin end is soon hydrolyzed to GDP, which weakens the binding affinity of tubulin for adjacent molecules and favors depolymerization[2]. A microtubule grows or shrinks depending on the rate of tubulin addition relative to the rate of GTP hydrolysis. If new GTP-bound tubulin molecules are added more rapidly than GTP is hydrolyzed, the microtubule retains a GTP cap at its plus end and microtubule growth continues. However, if the rate of polymerization slows, the GTP bound to tubulin at the plus end of the microtubule will be hydrolyzed to GDP, and the GDP-bound tubulin will dissociate, resulting in rapid shrinkage of the microtubule[2].

The dynamic instability of the microtubules is vital to the cell division cycle. During interphase of mitosis, microtubules are initially present in an array in which they

extend outward from a microtubule organizing center. Later during prophase, this array is disassembled and the free tubulin subunits rearrange to form the mitotic spindle. Chromosomes then attach to the microtubule spindle formation via their kinetochores and are separated during anaphase. Microtubules also play an important role in cytokinesis when the cell membrane and cell wall are separated equally between two daughter cells. Because microtubules play such a prominent role in cell division, a number of anti-cancer drugs inhibit tubulin polymerization to arrest rapidly dividing cells. Since microtubules are important to cell physiology, their physical properties and the effect they have on other cellular structures should be investigated.

Fission Yeast

In all our experiments, we worked with the fission yeast *Schizosaccharomyces pombe*, which is classified as an ascomycete fungus. The fungus was first described by the English scientist P. Linder in 1893. Linder isolated these organisms from an East African millet beet and chose the epithet p*ombe* for the Swahili word for beer. While *S. pombe* are in the same class as budding (baker's) yeast, RNA and DNA sequence analyses have shown that the fission yeast are as far removed in the phylogeny from human beings as they are from their budding yeast relatives. The *S. pombe* lineage separated around 1 billion years ago into a branch of ascomycete known as archaeascomycetes[5].

Fission yeast are used extensively in molecular and cell biology as model organisms due to their simplicity and availability. The entire genome of *S. pombe* was sequenced in 2002 by researchers at the Sanger Institute in Cambridgeshire, England, and nearly all of the organism's proteins have been localized. Fission yeast were used in cell

cycle regulation experiments by Paul Nurse, Lee Hartwell, and Tim Hunt, for which they were awarded the 2001 Nobel Prize in Physiology or Medicine. The yeast are simple to culture, and have a short generation time of approximately three hours. Since its whole genome has been sequenced, mutant forms of *S. pombe* are readily available and allow for a wide variety of experiments[5].

Atomic Force Microscopy

We examined the yeast cell walls using an atomic force microscope (AFM), an instrument designed by Binnig and Quate in 1986. Using an AFM to scan a sample can be compared to a blind person scanning his environment with a stick[9]. In AFMs used for biological applications, a computer monitors the deflection of a laser from a microscale cantilever. The cantilever, approximately 100-200μm in length, is made from silicon nitride and has a nanoscale stylus tip at its end. As this tip interacts with the sample, the laser's deflections are monitored with an optical detector at a resolution of less than 0.1 nm, allowing forces in the 10-50 pN range to be measured. After the stylus makes contact with the sample, a servo-system moves the sample vertically to maintain the force preset after the initial tip-sample approach. Precise lateral and vertical displacements are achieved using a piezo scanner in the AFM head. An AFM is capable of scanning the topography of a sample to collect an image, as well as taking force curves at specific points along the sample. In the Asylum AFM used in our research, there were two available imaging modes: contact and AC (tapping) mode. The difference between these modes depends on the interplay between the cantilever deflection detector and the servo-system that adjusts the cantilever's height. In contact mode, aka constant force mode, the cantilever retracts from or extends toward the sample depending on the polarity of the

cantilever deflection. In tapping mode, the cantilever continually oscillates up and down at its resonance frequency. The servo-system detects the reduction in this oscillation amplitude when contact is made between the tip and the sample. Because the oscillation amplitude is disrupted periodically, lateral forces on the cantilever are minimized. A schematic of the AFM's operational parts is shown in Figure 2.

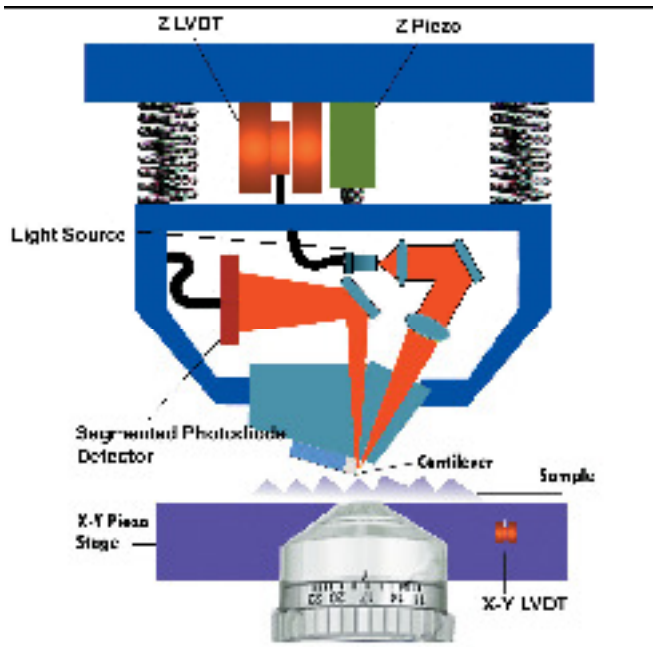


Figure 2 AFM Schematic[6]

In our experiments, we worked in contact mode to collect force curves along the yeast cell wall since the yeast adhered weakly to the substrate and oscillations from the cantilever would move them out of place. We collected several curves at a single point on the yeast cell, and also moved the tip to different points along the yeast cell using a Nudger software tool. We checked for consistency in the force measurements we made, looked for the variation in force at different points along the same cell, and checked for similarities in the mechanical properties of different cells.

Experimental Procedure

Background

The goal of our experiments was to quantify the basic mechanical parameters of the yeast cell wall using atomic force microscopy. We sought to culture healthy yeast cells according to standard procedures and subsequently immobilize them in order to perform force measurements. While hundreds of force-distance curves were collected, the majority exhibited substantial background noise, and only 30 curves were analyzed for the purpose of this thesis.

Cell Culture and Reagents

We conducted our experiments using the wild-type *Sacchyromyces pombe* strain DB558. We cultured the strain in sterile conditions using both rich and minimal media. Yeast Extract with Supplements (YES) Agar Powder and Edinburgh Minimal Media (EMM2) were obtained from US Biological. Instructions for preparing both media are given below.

YES Media Preparation

1. Dissolve 52.3 grams in approximately 900 mL of DI water with gentle stirring and heating until the mixture is completely solubilized.
2. Adjust the pH to seven.
3. Add additional water to bring the total volume of the solution to one liter.
4. Dispense the media into appropriate containers and autoclave the solution for fifteen minutes at 121^{o}C.

EMM2 Media Preparation

1. Dissolve 32.33 grams for every liter of distilled, deionized water with heat and gentle stirring.

2. Adjust the pH level to seven if necessary.
3. After dispensing the solution into appropriate containers, autoclave for 15 minutes at 121°C.

Both the YES and EMM2 can be sterilized using 0.2 µm filters instead of autoclaving, and we used both methods in our experiments. In addition to preparing media, it was necessary to make a media base for the fission of the *S. pombe* which allows for vegetative growth. The protocol for making this agar base is the same as that for making the agar media, except the liquid should be poured into a Petri dish immediately after autoclaving and allowed to harden. For all experiments, the fission yeast culture was generated by growing the cells for 36-72 hours at 30°C on YES agar plates (Q-Biogene; 4101-711). The cells were then stored in a 4°C fridge for up to three months.

Growth Suspension

In order to obtain healthy, viable cells for force experiments, we needed to remove the yeast from the agar and suspend them in liquid media. We generated a pre-culture and from that prepared an exponentially growing yeast cell culture. For the pre-culture, we transferred cells from the agar plates to a 50 mL centrifuge tube (Fisher Scientific; 14-375-150) containing 10 mL of YES media. The pre-culture should be set at an optical density (OD) of 0.2 when analyzed with 595 nm light, and should be grown until the suspension reaches an OD of 0.5. From this suspension, the yeast should be transferred to an Erlenmeyer flask containing about 20 mL of the relevant growth media. Since the final OD of the culture should be between 0.5 and 0.7, the volume of yeast added from the pre-culture must be back-calculated. The lifetime of the yeast is three hours at 25°C, and therefore the amount added from the pre-culture should depend on the amount of time the yeast are left to grow. To ensure the yeast cells used in experiments are healthy, the

culture should be allowed to grow overnight but always at an OD above .02. After the exponentially growing culture is set, the flask should be covered with aluminum foil and place in a 25°C shaking incubator.

We did not always follow the procedure outlined above in our experiments. When we first conducts these trials, we did not set a pre-culture before starting an exponentially growing culture, as we did not realize this was a standard practice. Furthermore, in later experiments we found that the yeast grew much more slowly than anticipated, sometimes taking over 6 hours to divide. Hence, the pre-cultures we used generally ended an OD of only 0.2, and the culture which started with an OD of 0.02 ended around 0.2 instead of 0.5. These difficulties with growing the yeast are most likely attributable to improper maintenance of the media. The manufacturer recommended keeping prepared media at 4°C, but in our experiments we stored media at room temperature under the cell culture hood.

Cove slip Preparation

The yeast cells were immobilized on 14 mm Menzel Glaser glass coverslips, which we cleaned using the following protocol:

1. Place the glass cover slips in a metal rack, and gently put the rack inside a sturdy glass holding container.
2. Fill the container with a 2% Hellmanex solution until the cover slips are completely immersed.
3. Cover the holding container and place it in the ultrasonic agitation bath for fifteen minutes.
4. Exchange the Hellmanex with distilled water and place the container for another fifteen in the ultrasonic agitator.

5. Pour out the water and add Hellmanex solution again, and repeat steps 3 and 4 two more times.

6. Empty the water from the container and dry the cover slips in the vacuum over at 70 C for two to three hours. Do not leave the cover slips in the oven overnight since this can cause contamination of the surface.

Yeast Cell Immobilization

In order to collect accurate force-indention curves using the atomic force microscope, it is imperative that the yeast cells remain stationary during scans. At the same time, the adhesion forces holding the cell to a coverslip should be minimal as to not affect the cell wall's natural elastic response. In practice, this balancing act between adhering cells to the coverslips and minimizing the forces used makes the sample problematically sensitive. While several alternatives were considered, all samples prepared in our experiments utilized the positively charged amino acid polymer Poly-L-lysine (Sigma Aldrich; 1399). Poly-L-lysine enhances electrostatic interactions between the yeast cell wall and the culture surface and thereby acts as a nonspecific attachment factor for the cells. Once adsorbed onto a glass surface, Poly-L-lysine increases the number of positive binding sites available to the cell.

To ensure the Poly-L-lysine would effectively adsorb to the glass, we used Hellmanex cleaned coverslips and plasma cleaned them prior to coating with the polymer. The protocols for plasma-cleaning and P-L coating are given below.

Plasma Cleaning

1. Place one to two glass coverslips on top of an overturned beaker in an empty glass desiccator, as shown in Figure 1.

2. Cover the outer rim of the desiccator's lid with vacuum grease and close to create an air-tight seal.
3. Reduce the pressure in the desiccator to 0.1 psi using a vacuum pump.
4. Place the desiccator in a microwave oven for thirty seconds, making sure to check for a bright violet illumination.
5. Remove the desiccator from the microwave oven, using thermal gloves if necessary, and carefully open the lid.

After plasma cleaning, we removed the coverslips and placed them on thin foam before coating the slips with Poly-L-lysine using the following protocol:

1. Add approximately 150 µL of P-L to the coverslips to ensure complete surface coverage.
2. After one hour, pipet off the excess P-L and wash twice with deionzed water.
3. After waiting around twenty minutes for the coverslips to dry, wash the coverslips twice with deionized water.

Once the coverslips were cleaned and coated with Poly-L-lysine, were glued the slips to the center of a glass microscope slide using epoxy glue. We coated the edge of the round coverslip with hydrophobic silica gel to prevent media from leaking onto the glass slide. Finally, we added yeast cells suspended in EMM2 at an optical density of 0.2 to the coverslip before conducting scans using the atomic force microscope.

Atomic Force Scans

We conducted force scans using an Asylum Molecular Force Probe (MFP-3D BIO) Microscope. Before probing a sample, we aligned the cantilever and calculated and its spring constant and deflection sensitivity. The protocols for these tasks are given below.

Figure 3 AFM Microscope

Getting Started

1. Load the cantilever in the fluid cell holder as shown in Figure 4. Tighten the screws until resistance is felt.
2. With the AFM head turned upside down, place the fluid-cell holder in the proper socket.
3. Place a glass microscope slide on the AFM's X-Y stage above the lens.
4. Turn the head over and position it above the slide, making sure the cantilever is several millimeters from the surface.
5. Turn on the power to the AFM and the computer in the order specified in the manual, and start the Igor Pro software.
6. After setting the microscope on SP, increase the light level and find the cantilever using the visual plane knobs.
7. Decrease the brightness to see light from the laser, and align the laser spot over the tip of the desired cantilever.
8. Observe the values in the Sum-Deflection Meter, maximizing the sum while keeping the deflection to a minimum.

Figure 4 Cantilever Holder w/ Mounting Base

Calibrating InvOLS (Inverse Optical Lever Sensitivity)

1. Click "Engage" in the Sum –Deflection Meter and lower the cantilever on to the glass slide. The Z-voltage should decrease from +150V to -70V as the tip makes contact with the surface.
2. Collect a Force plot by clicking "Continuous" under the Force tab in the Master Panel and lowering the tip onto the glass surface. Be sure to set the axes of the force graph to Deflection Volts vs. LVDT using the Force Channel Panel.
3. You should see a smooth linear region where the tip contacts the surface. On the graph, hit Ctrl + I and put the A and B cursors on this linear region, making sure both cursors are on the same part of the line.
4. Select Deflection from the "Set Sensitivity" popup under the Calculation tab of the Force tab of the Master Panel. This fits the region between the cursors to a straight line and calculates the InVOLS.

Calculating the Spring Constant

1. Set the Deflection to zero and click "Do Thermal" under the Thermal tab, and click "Stop Thermal" after the sample count reaches a few dozen.

2. Should see several narrow resonance peaks descending in height with increasing frequency.
3. Estimate the frequency of the largest resonance peak and type this value in the "Zoom Center" box. Enter a reasonable value (~5-10 kHz) in the "Zoom Width" box.
4. Click on "Fit Guess" and then "Try Fit," which should fit a curve to the largest frequency peak and give a value for the spring constant.

Loading the Sample

1. Remove the head from the base and turn it upside down so that the spring clip is exposed.
2. Remove the glass microscope slide and replace it with the yeast cell sample.
3. Place a drop of solution next to the cantilever chip, and use a syringe tip to gently wet the cantilever with that drop.
4. Return the head to its original position over the sample and ensure that there is a solid meniscus between the tip and the surface that covers the cantilevers.
5. Adjust the light source to maximize the **Sum** and zero the **Deflection**. This generally requires a 1/4 clockwise turn of the LDX knob.
6. Complete a Thermal Power Spectral Density (PSD) to ensure that the light source is being reflected off the cantilever. The peaks seen should be broader than when the Thermal PSD was collected in air.

Scanning the Sample

1. Switch from AC Mode to Contact Mode and click "Engage" on the Sum-Deflection Meter and lower the cantilever onto glass.
2. Under "Programming," click "Start User Panel" and open the Nudger macro.
3. Under the Force tab, and click on "Continuous." Set the axes of the force graph to Force vs. LVDT.

4. Using the Nudger tool, position the cantilever above the middle of a yeast cell and lower the cantilever onto the cell. Once reasonable force curves are seen, save the curves using an appropriate base name and suffix.
5. Under MFP Controls, open the Video Panel and capture an image of the cell being scanned. An example is shown in Figure 5.
6. Continue to move the cantilever down the middle axis of the cell in .5-1µm increments using the Nudger and collect Force curves and each point. Repeat this process for several cells, collecting images and saving them with the appropriate base names and suffixes.

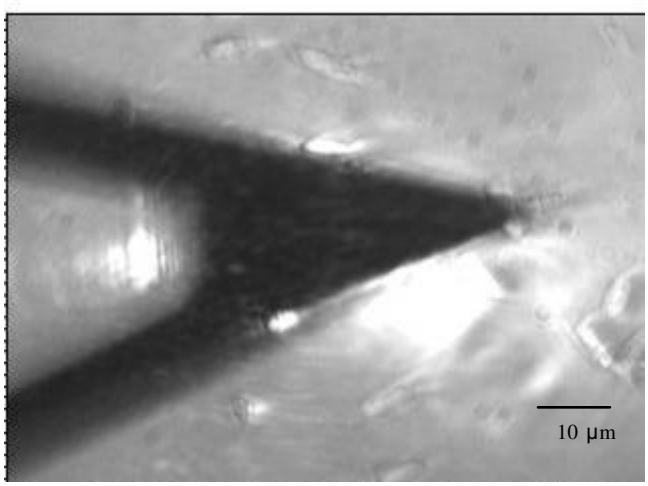
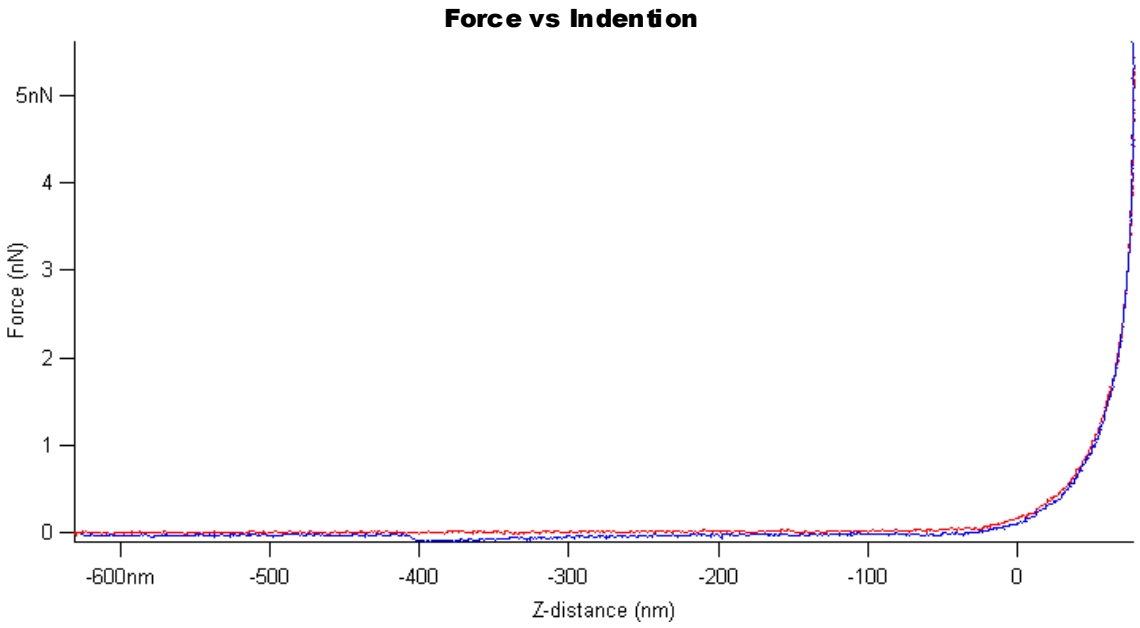


Figure 5 Cantilever Over a Yeast Cell

Experimental Data

The force vs. indentation curve we obtained for a representative yeast cell, which we labeled Yeast Cell 1, is shown below, along with a photograph showing where the cantilever tip was probing the cell wall.

A



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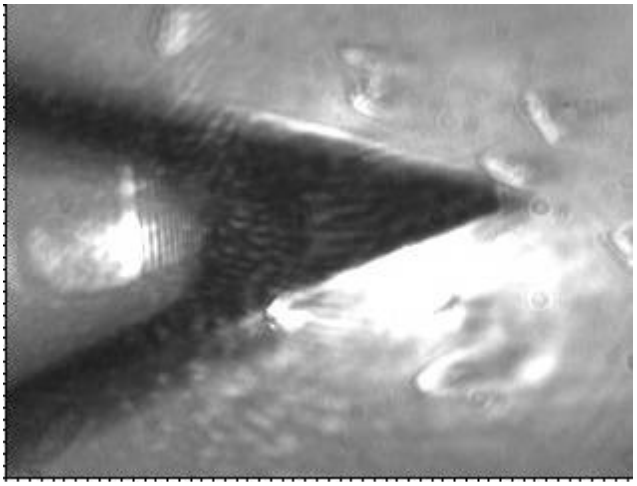


Figure 6 Cantilever Probing Yeast Cell Wall

(a) Force Extension Curve (b) Light micrograph of a cantilever over a yeast cell

We probed a single point on a yeast cell wall approximately 10 times and analyzed the force curves we collected to check the precision of our measurements. We fit the curves to an exponential model and then to the Hertz model. Using the Nudger tool in the MFP software, we were able to move the cantilever down the midline of a cell in 1µm increments, as shown in Figure 7.

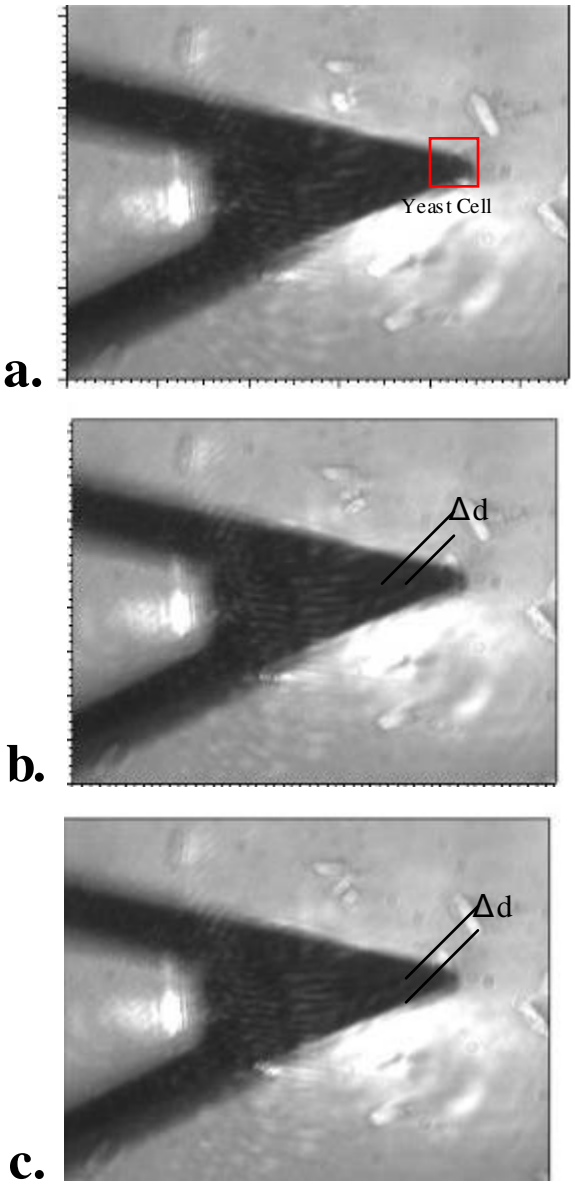
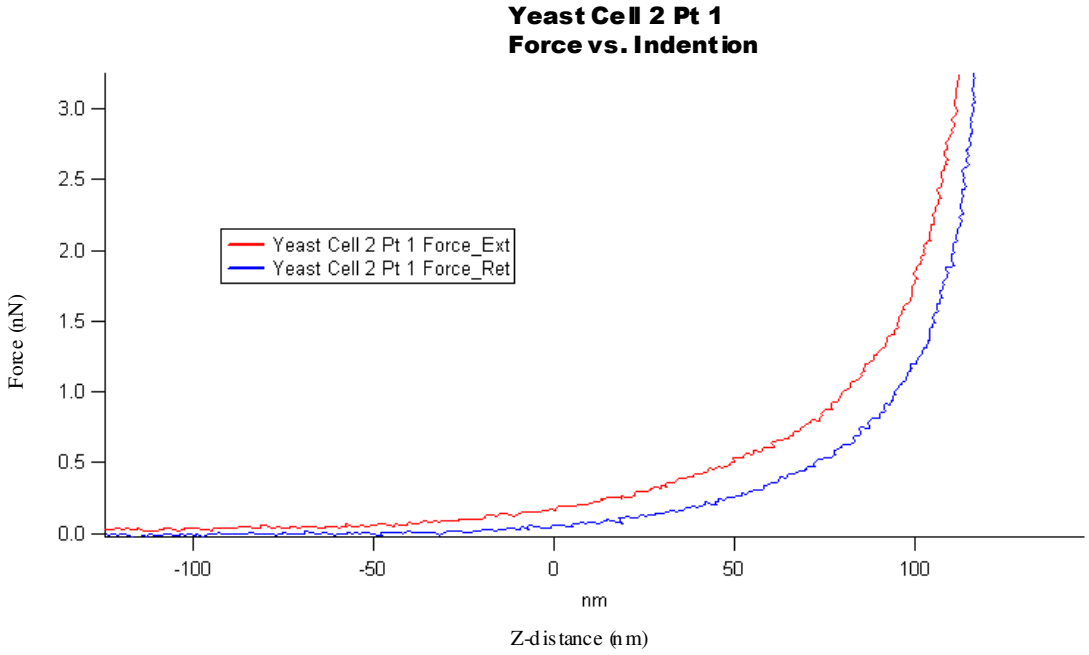
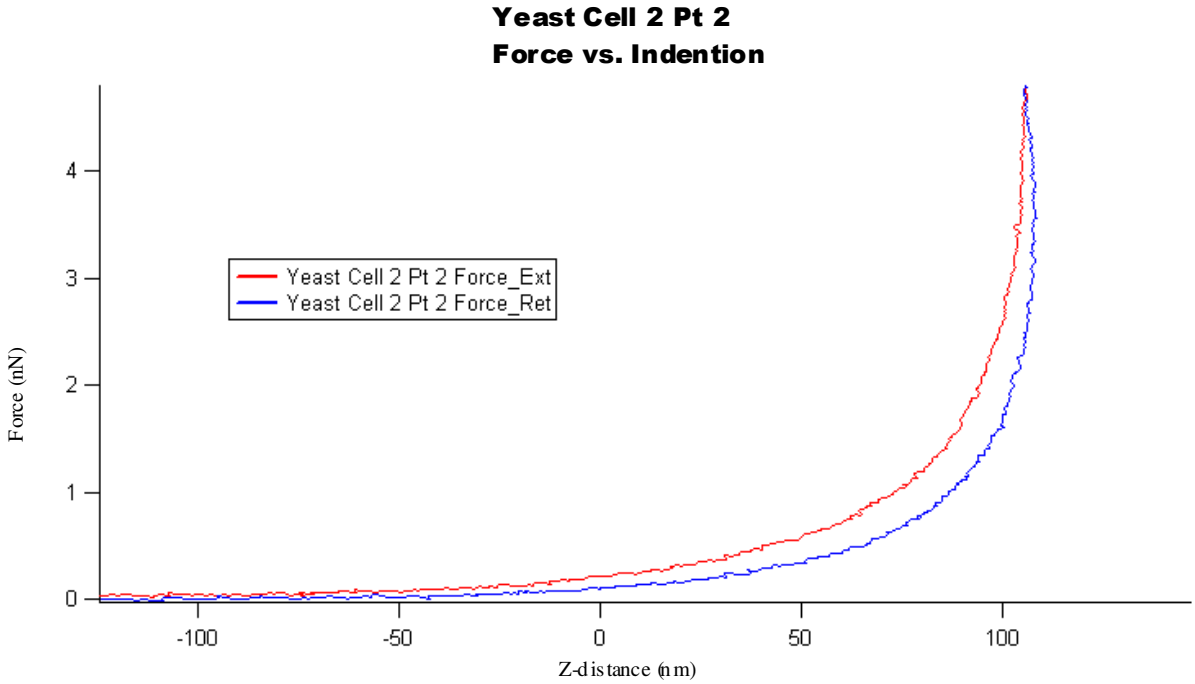


Figure 7 Cantilever Moving Down a Yeast Cell

(a) *Cantilever tip over a yeast cell (b) Tip displaced a distance Δd= 1µm down the midline of the cell (c) Cantilever moves another 1µm down the middle of the cell*

We collected multiple curves at each point and analyzed nine at each point to find the Young's Modulus and a characteristic length τ. Representative force graphs we collected while probing down the middle of the yeast cell in Figure 7 are shown below.





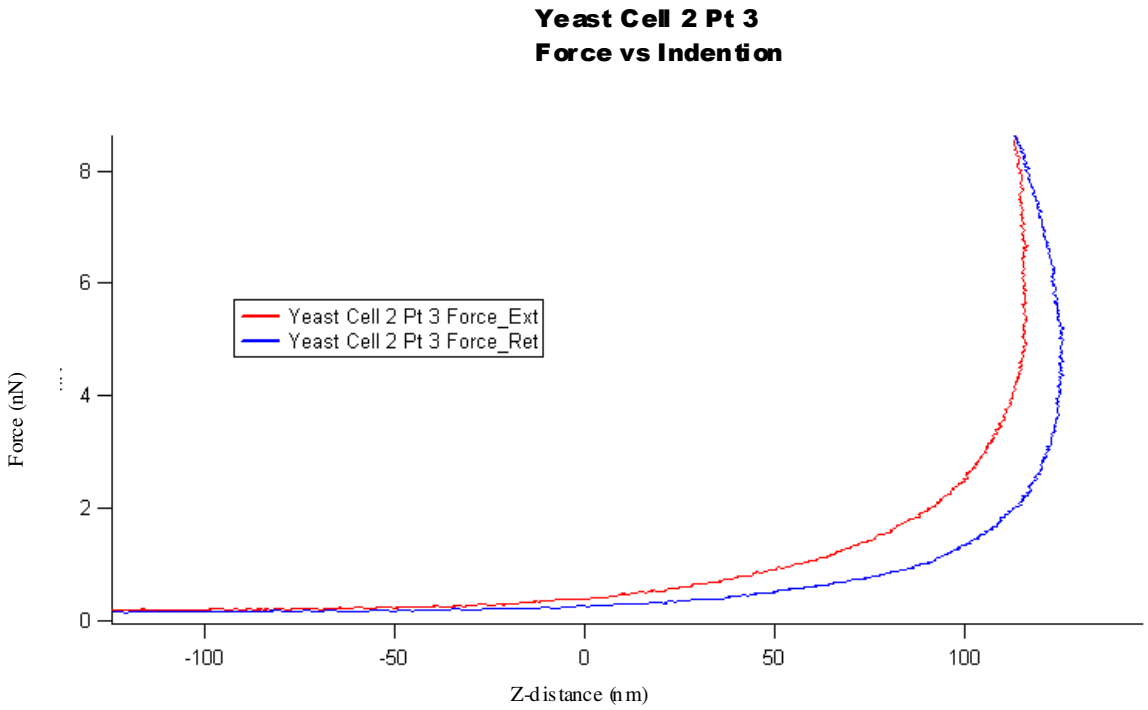


Figure 8 Force Curves Taken Along the Cell Wall

Shown above are three forces vs. indention curves corresponding to the cantilever positions shown in Figure 7. The extreme curvature of the force-retraction curve in the last graph is an artifact due to improper calibration.

After collecting force curves using the AFM, we set the X and Y offsets using the "Y offset" and "Raw Offset" options in Igor Pro, which automatically identifies where the cantilever first made contact with the sample. The Igor software defines the surface as the point where the cantilever deflection is the same as when the force curves start and labels this point as zero distance. Similarly, Igor Pro takes an average of ten Y values in each graph to offset the Y axis.

The experimental setup we used to examine the yeast cell wall presented many difficulties. Among others, we had problems getting the yeast cells to adhere to the poly-L-lysine coated glass coverslips used in our experiments. Movement of the cell upon cantilever approach led to excessive noise in the force curves obtained, which prevented us from fitting these curves to our models. We also ran into problems with the AFM

itself, as the equipment we used was outdated and could not perform many key tasks. Nevertheless, we were able to make due and collect many reasonable force curves.

Data Analysis

We analyzed several force curves to check for the reproducibility in the elastic response of the cell at a single point. Furthermore, we analyzed the response of the cell at different points along the same cell. Finally, we examined the variation in the elastic response of different cells at different points along the cell wall, and we also able to examine how different growth conditions affected the elastic response of the cell.

While we were expecting an approximately linear response from the cell wall, the curves we obtained show that the force is clearly nonlinear. Hence, we decided to fit our data to an exponential curve of the form $y = y0 + A*e^{((x-x0)/\tau)}$, where y0 and x0 are the y and x offsets, A is the normalization constant, and τ is a parameter called the "characteristic length", analogous to the characteristic lifetime in a decaying exponential. We analyzed our curves using Igor Pro 6.03 and MFP3D software version 07011+127. To ensure the validity of our fits, we set the program such that the fit coefficient confidence intervals were at a 99% confidence level. A graph of an exponential fit applied to a Force Extension vs. Indentation graph is shown below. For this particular graph, we found the following values for the fit coefficients using Igor Pro: A =1.92e-009 ± 1e-005, τ =2.53e-008 ± 4.9e-010m, x0=7.0e-008 ± 1.3e-4m, and y0=3.48e-011 ± 1.1e-011m.

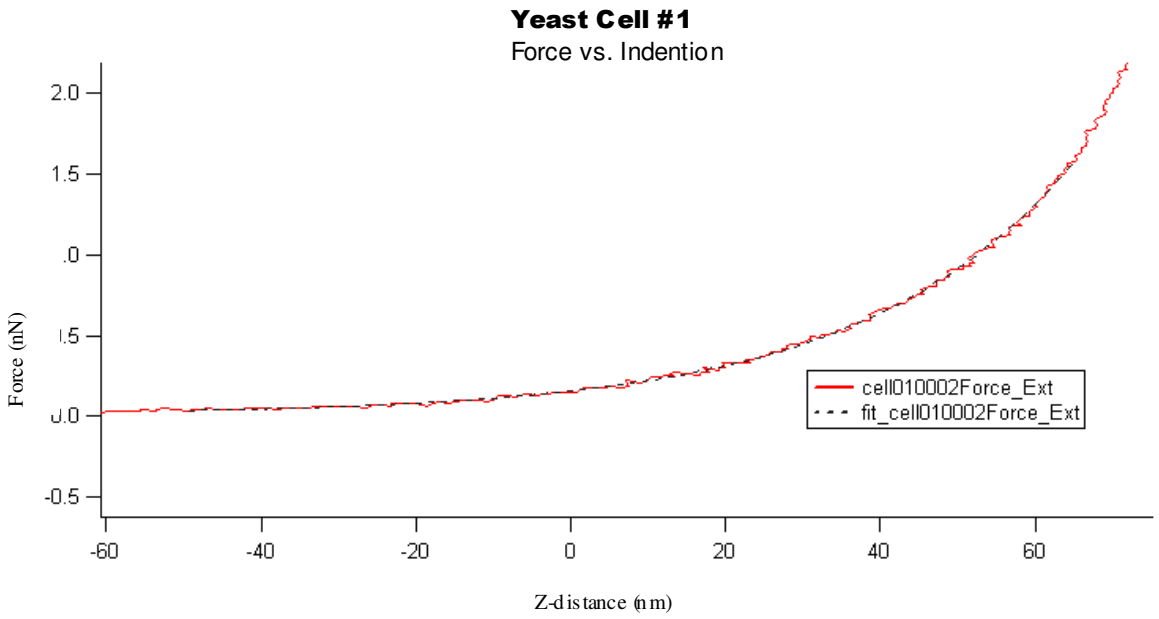


Figure 9 Exponential Fit to Force Extension Curve

We checked the validity of our characteristic length parameter τ by applying the exponential fit to multiple force curves taken at the same point along the cell wall. In every case, we fit the region between -50 and 65nm, where 0nm is the sample's surface. The values obtained are given in the table below.

Yeast Cell 1

Curve number	Characteristic Length τ (m)	Error in τ (m)
cell010001	2.61E-08	4.94E-10
cell010002	2.73E-08	4.69E-10
cell010003	2.59E-08	4.54E-10
cell010004	2.27E-08	5.74E-10
cell010005	2.64E-08	4.68E-10
cell010006	2.71E-08	5.04E-10
cell010007	2.67E-08	4.13E-10
cell010008	2.51E-08	4.75E-10
cell010009	2.68E-08	4.44E-10
Average	**2.60E-08**	4.77E-10
Standard Deviation	**1.40E-09**	

Next, we used the Hertz model, which was part of the Igor Pro software, to find an approximate value for the elastic moduli of the cell walls. This particular variant of the

Hertz model approximates the cantilever's tip as a rigid cone and the sample as a uniform elastic plane. Since yeast cell walls are composed of multiple polymer layers and are not necessarily uniform, the Hertz model does a poor job of accurately describing the cell. Consequently, this model did not fit the force data nearly as well as the exponential model we used. A representative curve fit with the Hertz model is shown below.

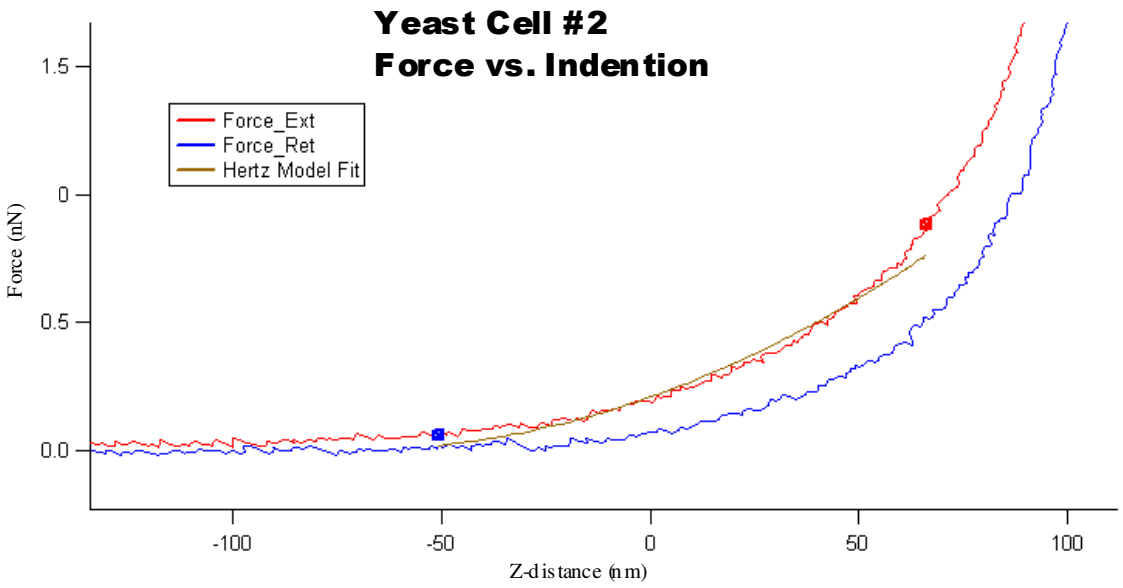


Figure 10 Hertz Model fit to a Force Extension Curve

A graph showing variation in the length parameter τ as force curves were taken along the mid axis of Cell 2 is shown below. The error bars on the graph represent the error in the τ coefficient reported by Igor after fitting to our exponential model.

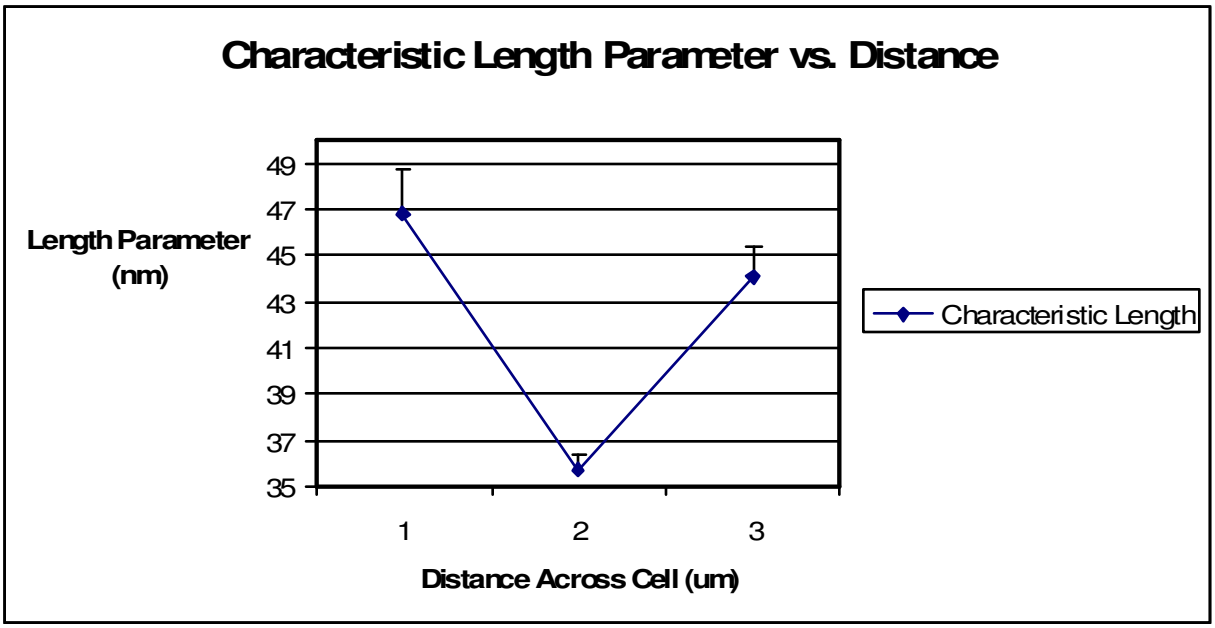


Figure 11 Variation in Characteristic Length with Relative Distance Across the Cell

The table below gives the average value for the length parameter τ that we found for each cell, and the average Young's Modulus we found using the Hertz model. For Cells 1 and 2, we analyzed 9 force curves at each point to obtain the values given in the table, and for Cells 3, 4, and 5 we analyzed 5 curves each.

Cell Number	Average Length τ (nm)	Error (nm)	Elastic Modulus (Pa)	Standard Deviation (Pa)
Yeast Cell 1	26.00	0.48	1.35E+07	6.54E+05
Yeast Cell 2 Pt 1	46.86	1.90	5.21E+06	3.56E+05
Yeast Cell 2 Pt 2	35.71	0.73	6.49E+06	4.65E+05
Yeast Cell 2 Pt 3	44.08	1.33	6.21E+06	3.16E+05
Yeast Cell 3	44.57	1.92	2.98E+04	8.58E+03
Yeast Cell 4	48.71	3.10	4.17E+04	2.61E+03

Yeast Cell 5	41.83	3.32	4.88E+04	9.74E+03
Average	**41.11**	**1.82**	**4.51E+06**	**2.59E+05**

As can be seen in the table above, the length parameter τ is roughly constant, to an order of magnitude, at all points. However, the elastic modulus is several orders of magnitude greater in Cells 1 and 2 than in Cells 3, 4 and 5. The only experimental difference between the two sets of cells is that Cells 3, 4, and 5 were grown to an optical density of .628A before they were seeded on the poly-L-lysine coated coverslips, while Cells 1 and 2 were only grown to an OD of .165A. The former is considered normal growth, while the latter is labeled undergrowth by cell biologists. The average elastic modulus of the normal growth cells was 7.9 ±4 MPa, while that of the undergrown cells was only 40.1 ±10 kPa. This is an interesting property of cell wall elasticity that we found depends on the cells' initial growth conditions. However, since our sample size is small, more tests will be needed to see if the difference in elastic moduli is a true distinction between undergrown and normally grown cells.

Conclusions

Though we were expecting linear force vs. indention graphs, our experiments showed that the elastic response of the yeast cell wall to stress is clearly nonlinear. Since the yeast cell wall is composed of several polymer layers, it cannot be simply modeled as a rigid structure. The Hertz model, which approximates the tip as a rigid cone and the sample as an elastic plane, is a poor representation of the yeast cell wall. Hence, the values of the Young's moduli we obtained are not necessarily reliable. However, we were able to fit exponential curves at a 99% confidence level to all the curves we obtained and found average values for the characteristic length parameter τ that were consistent among

all the curves. The average value of this parameter was 41.1± 7.9nm for all the curves we analyzed. These findings show that the elastic response of the yeast cell wall can be modeled using an exponential function in a roughly 100 nm range (from -50 to 65 nm). This response indicates that the outer layer of the yeast cell wall is likely a polymer brush. We believe that the characteristic length τ corresponds to the depth of this outer polymer layer constituting the cell wall. A rough representation of our model is shown in Figure 12.

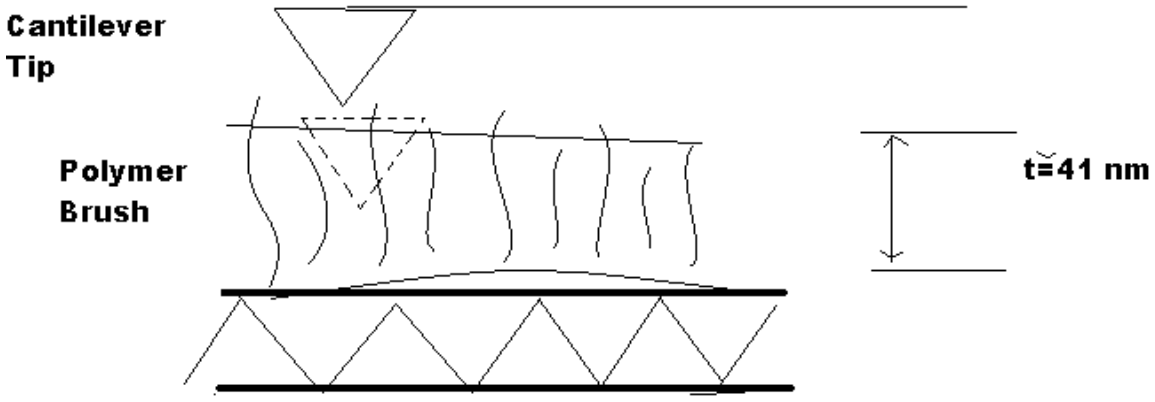


Figure 12 Cantilever Indenting Polymer Layer on Yeast Cell Wall

Through future experiments, we hope to obtain a larger sampling of yeast cells and quantify forces at many different points along the cell. We also hope to quantify the forces acting on the yeast cell during mitosis using fluorescent beads, which we would implant in the cell wall at a specific initial distance. If we have an accurate model for the cell's elastic response, we can calculate the forces acting on the cell by measuring the distance between the beads after mitosis. By continuing work on this project, we hope to obtain a better quantitative understanding of the forces exerted on a cell during its lifetime.

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